

Appendix 2
Entire Set Of Pending Claims

26. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products, comprising:

a) providing:

i) a cleavage agent;

ii) a source of target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;

iii) a first oligonucleotide, wherein a first portion of said first oligonucleotide comprises at least one nucleotide analog and wherein said first portion is completely complementary to said first portion of said first target nucleic acid, and;

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second region of said target nucleic acid;

b) combining said cleavage agent, said target nucleic acid, said first oligonucleotide and said second oligonucleotide under reaction conditions such that at least said first portion of said first oligonucleotide is annealed to said first region of said target nucleic acid, and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs and cleaves said first oligonucleotide to generate a non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

27. The method of Claim 26, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

28. The method of Claim 26, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

29. The method of Claim 27, wherein said 3' terminal nucleotide not complementary to said target nucleic acid comprises a nucleotide analog.

30. The method of Claim 28, wherein said single nucleotide not complementary to said target nucleic acid comprises a nucleotide analog.

31. The method of Claim 26, wherein said target nucleic acid comprises a nucleotide analog.

32. The method of Claim 31, wherein said first region of said target nucleic acid comprises said nucleotide analog.

33. The method of Claim 26, wherein said cleavage of said cleavage structure cleaves said first oligonucleotide.

34. The method of Claim 27, wherein said cleavage of said first cleavage structure cleaves in said first portion of said first oligonucleotide.

35. The method of Claim 27, wherein said first oligonucleotide is cleaved 5' of a nucleotide analog.

36. The method of Claim 35, wherein said first oligonucleotide is cleaved adjacent to and 5' of a nucleotide analog.

37. The method of Claim 27, wherein said first oligonucleotide is cleaved 3' of a nucleotide analog.

38. The method of Claim 37, wherein said first oligonucleotide is cleaved adjacent to and 3' of a nucleotide analog.

39. The method of Claim 27, wherein said first oligonucleotide further comprises a second portion, wherein said second portion is 5' of said first portion, and wherein said second portion comprises at least one nucleotide analog.

40. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.

41. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

42. The method of Claim 26, wherein said first oligonucleotide comprises a fluorophore having quenched emission, and wherein said detecting the cleavage of said cleavage structure comprises detection of an increase in fluorescence intensity.

43. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, dye intercalation, fluorescence polarization, staining, or color.

44. The method of Claim 26, wherein said first oligonucleotide is attached to a solid support.

45. The method of Claim 26, wherein said second oligonucleotide is attached to a solid support.

46. The method of Claim 26, wherein said target nucleic acid comprises synthetic nucleic acid.

47. The method of Claim 46, wherein said synthetic target nucleic acid comprises an amplified nucleic acid.

48. The method of Claim 47, wherein said amplified nucleic acid is produced using a polymerase chain reaction.

49. The method of Claim 46, wherein said synthetic target nucleic acid comprises DNA.

50. The method of Claim 26, wherein said cleavage agent comprises an enzyme.

51. The method of Claim 50, wherein said enzyme comprises a DNA polymerase.

52. The method of Claim 51, wherein said DNA polymerase comprises a thermostable DNA polymerase.

53. The method of Claim 52, wherein said thermostable DNA polymerase is derived from an organism from genus *Thermus*.

54. The method of Claim 53, wherein said organism from genus *Thermus* is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

55. The method of Claim 50, wherein said enzyme comprises a 5' nuclease.

56. The method of Claim 50, wherein said enzyme comprises a thermostable 5' nuclease derived from a thermostable DNA polymerase modified to have reduced synthetic activity.

57. The method of Claim 56, wherein said thermostable DNA polymerase modified to have reduced synthetic activity is derived from an organism from genus *Thermus*.

58. The method of Claim 57, wherein said organism from genus *Thermus* is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

59. A method of detecting a target nucleic acid, comprising:

a) providing:

- i) a cleavage agent;
- ii) a sample suspected of containing a target nucleic acid;
- iii) a first oligonucleotide comprising a 5' portion complementary to a first region of said target nucleic acid; and
- iv) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion;

wherein at least one of said first oligonucleotide, said second oligonucleotide or said first or said second regions of said target nucleic acid comprises a nucleotide analog;

b) combining said cleavage agent, said sample suspected of containing a target nucleic acid, said first oligonucleotide and said second oligonucleotide under reaction conditions such that at least said first portion of said first oligonucleotide is annealed to said first region of said target nucleic acid, and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate a non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

60. A kit comprising:

- i) a cleavage agent;

- ii) a first oligonucleotide comprising a 5' portion complementary to a first region of a target nucleic acid; and
 - iii) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion;
- wherein at least one of said first oligonucleotide, said second oligonucleotide or said target nucleic acid comprises a nucleotide analog.

61. The kit of Claim 60, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

62. The kit of Claim 60, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

63. The kit of Claim 54, wherein said kit further comprises a solid support.

64. The kit of Claim 63, wherein said first oligonucleotide is attached to said solid support.

65. The kit of Claim 63, wherein said second oligonucleotide is attached to said solid support.

66. The kit of Claim 60, wherein said cleavage agent comprises a structure-specific nuclease.

67. The kit of Claim 66, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

68. The kit of Claim 60, wherein said cleavage agent comprises a 5' nuclease.

69. The kit of Claim 68, wherein said 5' nuclease comprises a thermostable 5' nuclease.

70. The kit of Claim 66, wherein a portion of the amino acid sequence of said structure-specific nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

71. The kit of Claim 70, wherein said thermophilic organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

72. The kit of Claim 66, wherein said structure-specific nuclease comprises a FEN-1 endonuclease.

73. The kit of Claim 60, further comprising a buffer solution.

74. The kit of Claim 73, wherein said buffer solution comprises a source of divalent cations.

75. The kit of Claim 74, wherein said divalent cation is selected from the group consisting of Mn^{2+} and Mg^{2+} ions.

76. The kit of Claim 60, further comprising providing a third oligonucleotide complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid.

77. The kit of Claim 60, further comprising a target nucleic acid.

78. The kit of Claim 77, further comprising a second target nucleic acid.

79. The kit of Claim 78, further comprising a third oligonucleotide comprising a 5'

portion complementary to a first region of said second target nucleic acid.

80. The kit of Claim 79, wherein said 3' portion of said third oligonucleotide is covalently linked to said second target nucleic acid.

81. The kit of Claim 79, wherein said second target nucleic acid further comprises a 5' portion, wherein said 5' portion of said second target nucleic acid is said third oligonucleotide.

82. A method for detecting a target sequence, comprising cleaving a cleavage structure with a cleavage agent to generate a labeled cleavage product, wherein said cleavage structure comprises:

- g) a first nucleic acid molecule comprising a first region and a second region, said first region upstream of said second region;
- h) a second nucleic acid molecule that is complementary to said first region, said second nucleic acid molecule having a 3' end that is not extendable by a polymerase when said second nucleic acid molecule is hybridized to said first nucleic acid molecule; and
- i) a third nucleic acid molecule that is complementary to said second region, said third nucleic acid molecule comprising a label, wherein said labeled cleavage product comprises said label following cleavage of said cleavage structure.

83. The method of Claim 82, wherein said cleavage agent comprises a 5' nuclease.

84. The method of Claim 83, wherein said cleavage agent comprises a thermostable 5' nuclease.

85. The method of Claim 84, wherein said thermostable 5' nuclease comprises Taq polymerase.

86. The method of Claim 82, wherein said first, second, or third nucleic acid molecule

comprises a nucleotide analogue.

87. The method of Claim 82, wherein said 3' end that is not extendable by a polymerase comprises a nucleotide that the is not complementary to said first nucleic acid molecule when said second nucleic acid molecule is hybridized to said first nucleic acid molecule.

88. The method of Claim 82, wherein said first region and said second region of said first nucleic acid molecule are separated from each other by at least one nucleotide distance.

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